Narumi NAKATO* & Kunio MITUI**: **Intraspecific polyploidy in *Diplazium subsinuatum* (Wall.) Tagawa**

中藤成実*・三井邦男**： ヘラシダの種内倍数性について

(Plate III-VI)

Diplazium subsinuatum is distributed from Ceylon (Sri Lanka) to Japan, through India, Thailand, the Phillippines, China, Taiwan and Korea (cf. Nakaike, 1975). In Japan, it is generally found on moist slopes and banks along streams in lowland forests of subtropical and warm temperate regions.

The cytological study on this species has been made by the senior author (1965), who observed the 123 bivalents at meiosis and the normal complement of 64 spores per sporangium in the plant from Kanagawa Pref., and he concluded that the species was a sexual hexaploid based on x=41. On the other hand, Momose (1967) reported apogamous plants from Hiroshima Pref., from embryological studies. Furthermore, Kawasaki (1960) reported two types of spore ornamentation in this species, and Satake (1972) also observed several types of spore morphology. From these observations, it is presumed that *D. subsinuatum* is cytologically and morphologically not a monotypic one. To clarify this problem, the present authors studied the chromosome numbers, spore characters and other features in Japanese specimens collected from 17 localities.

Materials and Methods

The localities of the plants used are listed in Table 1 with the cytological results. Gametic chromosomes were observed in spore mother cells which were fixed in Carnoy's fluid followed by the usual aceto-carmine squash method. Somatic chromosomes were observed in the cells of root tip treated in 0.002 M 8-hydroxyquinoline solution for 6 hours. The root tips were fixed in 45% acetic acid for 20 minutes, hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (2:1) at 60°C for 5 minutes, and squashed on slides.

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Aceto-orcein or aceto-gentian violet was used for staining. Dry, nonacetolyzed spores were coated with gold for the scanning electron microscope. The voucher specimens are deposited in the herbarium of Nippon Dental University.

Observations

Chromosome numbers: Chromosome numbers were determined on 18 plants from 18 localities as shown in Table 1. It was confirmed that there exist intraspecific polyploids, tetraploids, pentaploids and hexaploids, based on $x=40$ in this species. As stated above, Mitui (1965) previously studied a plant from Zinmuzi, Kanagawa Pref. and reported that it was hexaploid with $n=123$. But in the present study, a plant collected from the same place was reexamined and proved to be hexaploid giving the exact counts of $n=120$ and $2n=240$ (Plate III). Since the previous count was based on an incomplete preparation, it should be corrected to $n=120$. Meiosis was

Tab. 1. Locality and chromosome number of specimens used in the present study.

Specimen No.	Locality	Chromosome number
1	Yunotsuru, Kumamoto Pref.	$n=80$, $2n=160$
2	Iwayasan, Nagasaki Pref.	$2n=160$
3	Otokoyama, Kyoto Pref.	$n=80$, $2n=160$
4	Asuka, Nara Pref.	$2n=160$
5	Ashikubo, Shizuoka Pref.	$2n=160$
6	Izumo, Shimane Pref.	meiosis irreg., $2n=200$
7	Kuki, Mie Pref.	$2n=200$
8	Aziro, Shizuoka Pref.	$2n=200$
9	Yugashima, Shizuoka Pref.	meiosis irreg., $2n=200$
10	Kawazu, Shizuoka Pref.	$2n=200$
11	Mitsuishiyama, Chiba Pref.	$2n=200$
12	Kiyosumiyama, Chiba Pref.	meiosis irreg., $2n=200$
13	Isl. Iriomote, Okinawa Pref.	$2n=240$
14	Isl. Amamioshima, Kagoshima Pref.	$2n=240$
15	Isl. Yakushima, Kagoshima Pref.	$2n=240$
16	Izumo, Shimane Pref.	$n=120$, $2n=240$
17	Zinmuzi, Kanagawa Pref.	$n=120$, $2n=240$
18	Isl. Miyake, Tokyo Pref.	$2n=ca.\ 240$

observed in 2 tetraploids, 3 pentaploids and 2 hexaploids. Both the tetraploids and the hexaploids showed normal meiosis with 80 and 120 bivalents, respectively. On the contrary, the pentaploids showed irregular meiosis with many univalents, and an accurate analysis of chromosomes was carried out in a plant (No. 6), showing 70 bivalents and 60 univalents (Plate IV).

Spore character: The spore formations were examined in 13 plants (No. 1, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 17, 18). Both of the tetraploids and hexaploids had the normal complement of 64 spores in each sporangium. On the other hand, the pentaploids produced many abnormal spores with considerable variation in spore size, although well-developed spores were rarely present. Additionally it was noticed that the cytological differences were well expressed in ornamentation of spores. The spores of the tetraploids were covered with irregular series of ridges which form a membranous structure in the SEM micrographs (Plate V). By contrast, the spores of the hexaploids were covered with processes of various size and shape (Plate V), and the well-developed spores of the pentaploids had ornamentations of various degrees between those of the tetraploids and the hexaploids (Plate VI). The differences in the spore ornamentation among the three cytotypes could be clearly observed even under the optical microscope.

Fifty spores (mounted in Canada balsam) per specimen were measured in the tetraploids and the hexaploids (No. 1, 4, 5, 13, 14, 15, 17, 18). The results

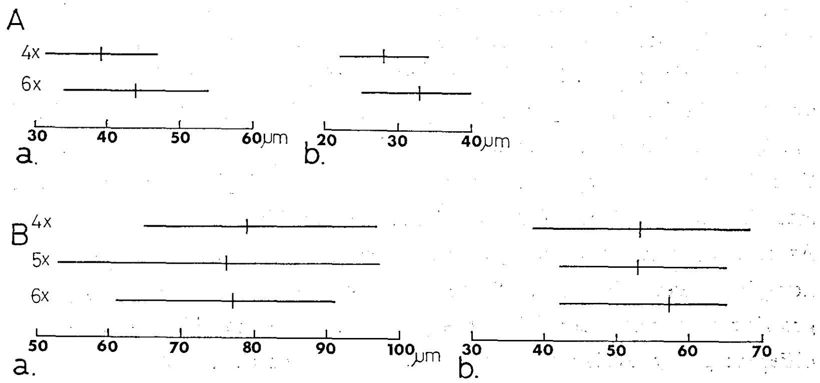

Fig. 1. Ranges and means of sizes of spore and stomata. A. Spore size. a, longest equatorial diameter. b, polar diameter. B. Stomata size. a, length. b, breadth.

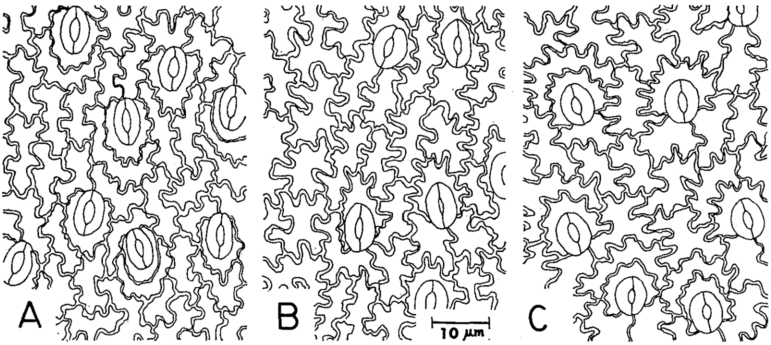

Fig. 2. Epidermis of three cytotypes. A. Tetraploid (Specimen No. 1). B. Pentaploid (No. 10). C. Hexaploid (No. 17).

are shown in Fig. 1, A. It is found that mean spore-size of the hexaploids (43.8 μm × 32.6 μm) was slightly larger than that of the tetraploids (39.3 μm × 28.3 μm).

Epidermis: The samples of epidermis were taken from the middle portion of the abaxial surface of the fertile frond and are shown in Fig. 2. The epidermal cells were variable in outline and size, and seemed to have no value in segregating the cytotypes. The size of stomata was measured in 50 samples per specimen in all materials and the results are indicated in Fig. 1, B. The mean size was 79.0 μm × 53.4 μm in the tetraploids, 76.3 μm × 52.7 μm in the pentaploids and 76.9 μm × 56.4 μm in the hexaploids. It seems that the cytological differences are not reflected in the size of stomata.

Distribution: Fig. 3 indicates the geographical distribution of the three cytotypes in Japan. A particular trend of distribution is not found in any cytotypes as far as the present study is concerned. In addition, it seems that the three cytotypes have not any specific ecological requirement. All materials were collected from the similar moist habitats under lowland forests. As to the plants from Izumo, Shimane Pref., two cytotypes, pentaploid and hexaploid, were detected. Since the two clones of these cytotypes were growing as a mixed condition, the present authors consider that plants at different ploidy levels may occasionally grow sympatrically in certain habitats.

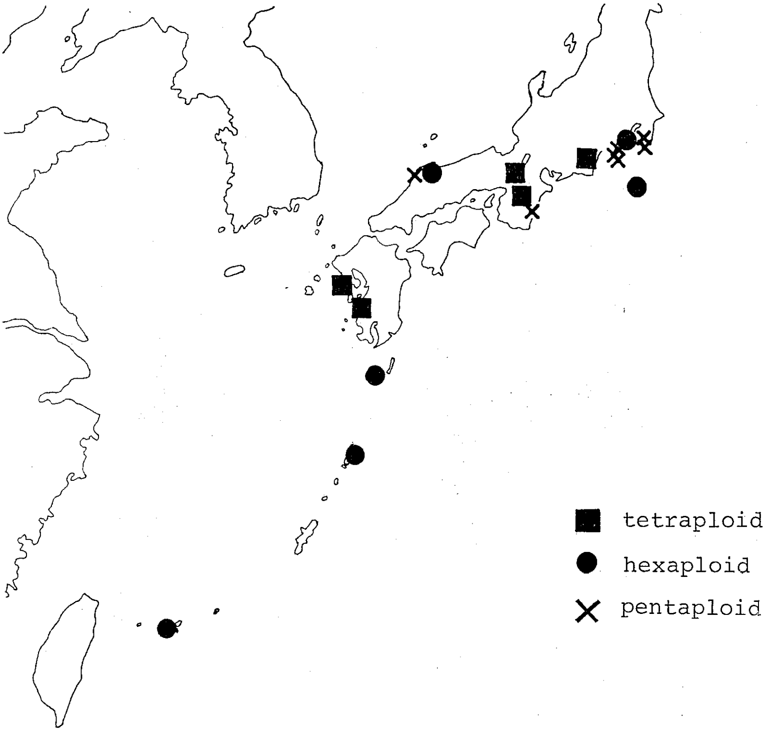

Fig. 3. Distributional map of three cytotypes in *Diplazium subsinuatum*.

Discussion

The present study shows that *D. subsinuatum* forms a polyploid series of the three cytotypes, 4x, 5x and 6x based on x=40 in Japan. The finding of this number is of great interest, because other species of *Diplazium* cytologically studied are based on x=41 without exception (Löve et al., 1977). From the present observation, the tetraploid and hexaploid were sexual in reproduction, and we did not find apogamous plants as observed by Momose (1967).

In external morphology the plants of three cytotypes resemble one another closely, and noticeable differences which may be useful in distinguishing the cytotypes, could not be detected. It is well known that in ferns where intraspecific polyploidy is present, the higher grade of ploidy is accompanied by a relative increase in the sizes of stomata and spores. Such examples hitherto reported on Japanese ferns are as follows: *Cyrtomium falcatum* (L.f.) Pr. (2x, 3x, 4x), *Dryopteris sparsa* O.Ktze. (2x, 3x, 4x), *D. yabei* Hayata (2x, 3x), *Lepisorus thunbergianus* (Kaulf.) Ching (2x, 3x, 4x), *Leptorumohra miqueliana* (Maxim.) H.Ito (2x, 3x, 4x), *Phegopteris decursivepinnata* (van Hall) Fée (2x, 3x, 4x), *Pteris excelsa* Gaud. (2x, 3x) and *P. fauriei* Hieron. (2x, 3x) (Hirabayashi, 1969, 1974; Mitui, 1966, 1968, 1976; Nakato, 1976; Yasuda, 1977). In the *D. subsinuatum* complex, the spore size is slightly larger in the hexaploids than in the tetraploids. However the size of stomata is apparently not correlated with ploidy levels as shown in Fig. 1, B. This trend may be partly due to the high grade of ploidy in this species (4x, 5x, 6x). In a comparative study on gigantism in many polyploid plants, Takenaka (1947) proposed that, although the size of stomata and others increased in accordance with the higher ploidy level, the gigantism was not attained when ploidy levels were higher than pentaploid or so.

Kawasaki (1960) reported that in this species (under the name of *D. lanceum*) there were two types of spore ornamentation; one type based on a specimen from Kii (Wakayama Pref.) was B3 type in his system which had long processes on spore surface, and another type observed in material from Tosa (Kochi Pref.) belonged to his C2 type which had yellow wings. Furthermore, Satake (1972) observed spores of some plants from Shizuoka Pref., and reported that there were several types of spores, that is, spiny type (regular in spore size), club-shaped (reg. or irreg.), membranous (irreg.) and a mixture of the latter two (reg. or irreg.). In the present study it is found that spore morphology provides useful characters to separate the three cytotypes. Thus, the pentaploids are easily distinguished from the others by the production of many abnormal spores, while the tetraploids and the hexaploids produce normal spores, and the ornamentation is membranous in the former races, but processed in the latter. Judging from the figures presented by Kawasaki (1960), it seems that spores of his B3 type in this species corresponded to those of the present hexaploids and spores of C2 type corresponded to those of the tetraploids. Likewise, the spores of spiny or regular club-shaped ornamentations observed by Satake (1972) seem to be of hexaploids, and the other irregular-sized spores to be of pentaploids.

The basic chromosome number is considered to be an important character in the definition of the two genera, *Athyrium* ($x=40$) and *Diplazium* ($x=41$), since Manton and Sledge first drew attention to it in 1954. According to chromosome numbers, *D. subsinuatum* seems to be related to *Athyrium* and *Lunathyrium* having $x=40$ rather than to *Diplazium* with $x=41$. It is very interesting that Serizawa (1972) and Kato (1975) pointed out that the structure of groove of rachis in this species corresponded to that of *Lunathyrium* ($x=40$) rather than that of *Diplazium* ($x=41$).

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Explanation of plates III-VI

Plate III. Chromosomes of tetraploid and hexaploid. A. Somatic chromosomes of tetraploid ($2n=160$, specimen No.1). B. Gametic chromosomes of tetraploid ($n=80$, specimen No.3). C. Somatic chromosomes of hexaploid ($2n=240$, specimen No.17). D. Gametic chromosomes of hexaploid ($n=120$,

specimen No.17).

Plate IV. Chromosomes of pentaploid. A. Somatic chromosomes of pentaploid ($2n=200$, specimen No.10). B. Meiosis in the pentaploid race showing bivalents and univalents (specimen No.6). C. Explanetory diagram to B.

Plate V. Spores of tetraploid and hexaploid. A-D. Tetraploid. E-H. Hexaploid.

Plate VI. Spores of pentaploid.

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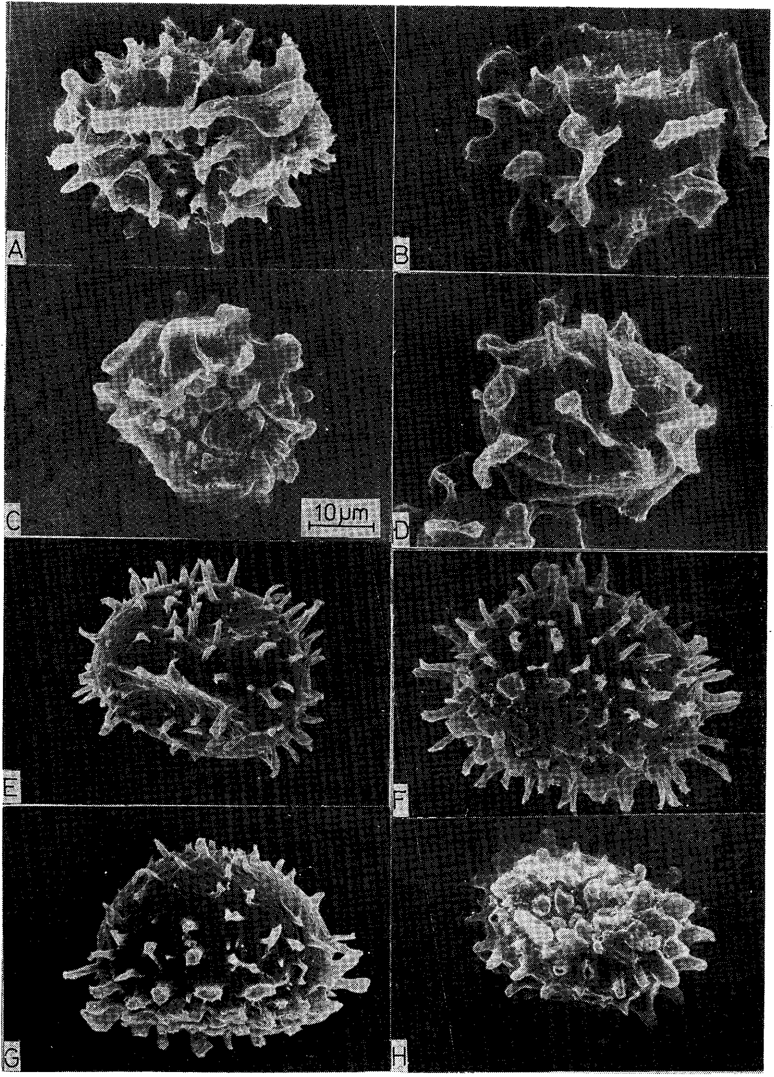
日本産のヘラシダを17地域から採集し，染色体数，胞子，表皮細胞について観察した。その結果，この種には $x=40$ に基づく4倍体，5倍体，6倍体の種内倍数体が存在することが明らかになった。4倍体と6倍体はともに有性生殖を行うが，5倍体では胞子の形成過程が正常ではなく，多くの不稔性とおもわれる胞子が観察された。5倍体の減数分裂ではおよそ70個の2価染色体と60個の1価染色体が観察された。

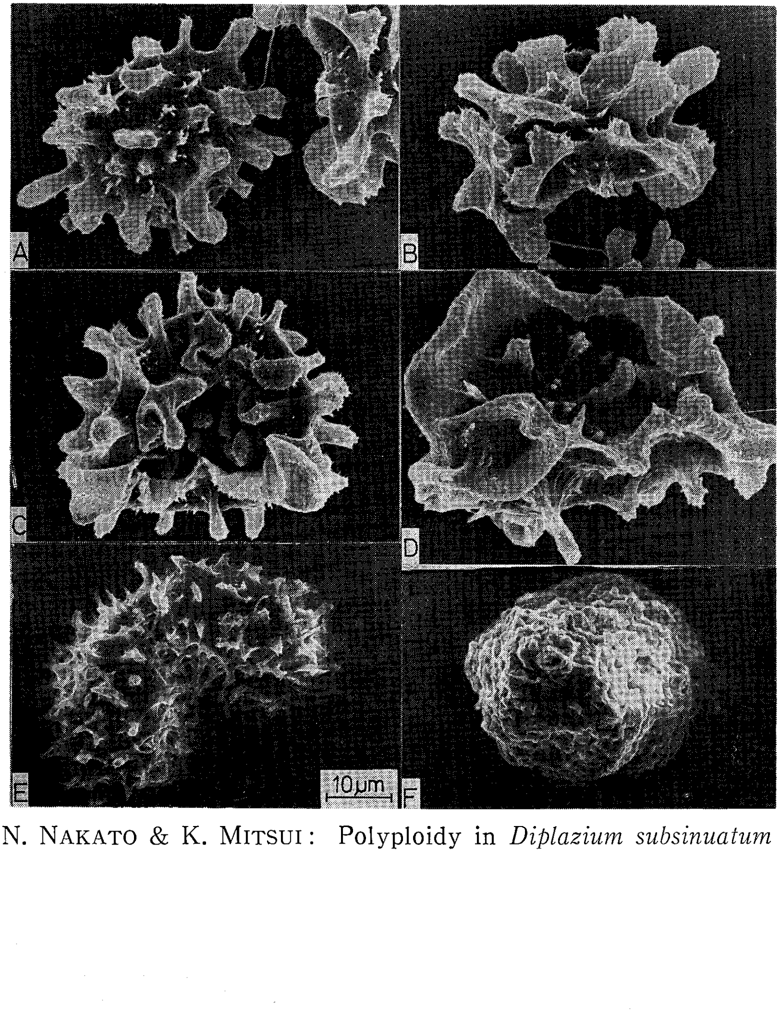
現在までに，多くのシダ類で種内倍数体が報告されている。そして一般的には，倍数性の増加にともない胞子の大きさ，表皮細胞の大きさの増加が認められているが，ヘラシダの種内倍数体間にはそのような顕著な傾向はみられなかった。

従来の報告ではヘラシダ属のものは $x=41$，メシダ属に属するものは $x=40$ に基づく染色体数を持っているが，今回の研究結果から，この2属を染色体基本数の点で区別することは不可能になった。

□山中二男：**高知県の植生と植物相**　461pp. 1978. 林野弘済会高知支部. ¥6,000. 高知大学教育学部の山中教授は30余年間高知県の植物の調査研究を続けておられる方で，植生に関する論文をたくさん発表している。その成果を極めてわかりやすく整理して述べたものが本書の前半で，後半は高等植物目録となっている。各府県の植物誌が出ている中で高知県にはまだそういうものがなかったところ，本書の出現で一躍その仲間入りができたわけであるが，内容，表現ともに独特のものを持っているのは喜ばしい。最初に本県植物の研究史と自然環境。次の「植生と植物地理」の内容は：森林帯と極相，植生の分類，暖温帯林，推移帯林，冷温帯林，亜寒帯林，海岸林，山地針葉樹林，超塩基性岩地帯の植生，石灰岩地帯の植生，渓谷林，川岸低木群落，二次林，草原，湿原・水生植物群落，海浜荒原，岩上荒原，耕作地，村落・市街地，竹林・人工林，まとめ，となっている。さらに「保護」の部には：歴史的経緯，天然記念物，保護林（14ケ所），自然休養林・風景林など，自然公園，未保護地域，社寺林，二次林および草地，保護の対象となる植物，の項目がある。植物目録はシダ植物と種子植物のそれで，分類順に挙げ名称のほかに生態環境と産地を要領よく記載してある。最後に文献，地名一覧，索引などが完備されている。高知県の植物を調べるのには欠かせない書物であると思う。なお，高知市丸ノ内1-3-30，林野弘済会高知支部に申し込めば送料込み上記の値段で入手できる。（伊藤　洋）



N. NAKATO & K. MITSUI: Polyploidy in *Diplazium subsinuatum*



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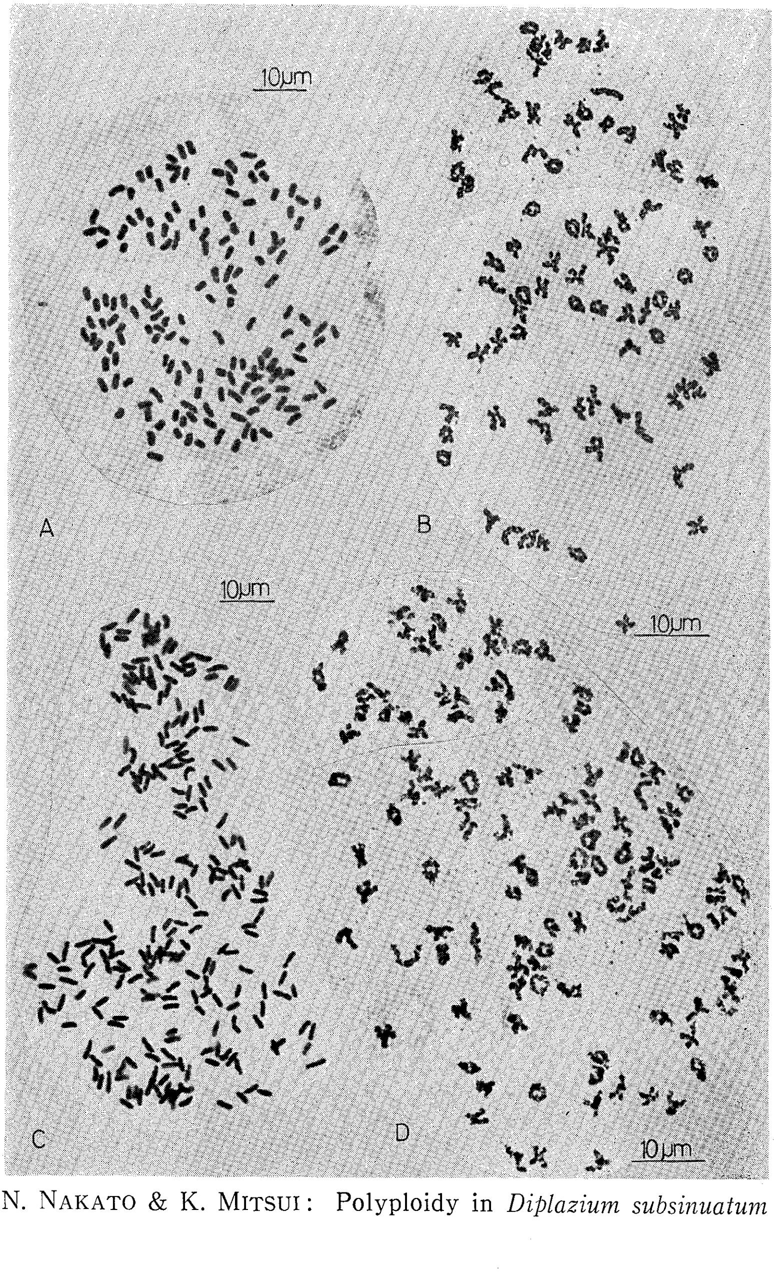

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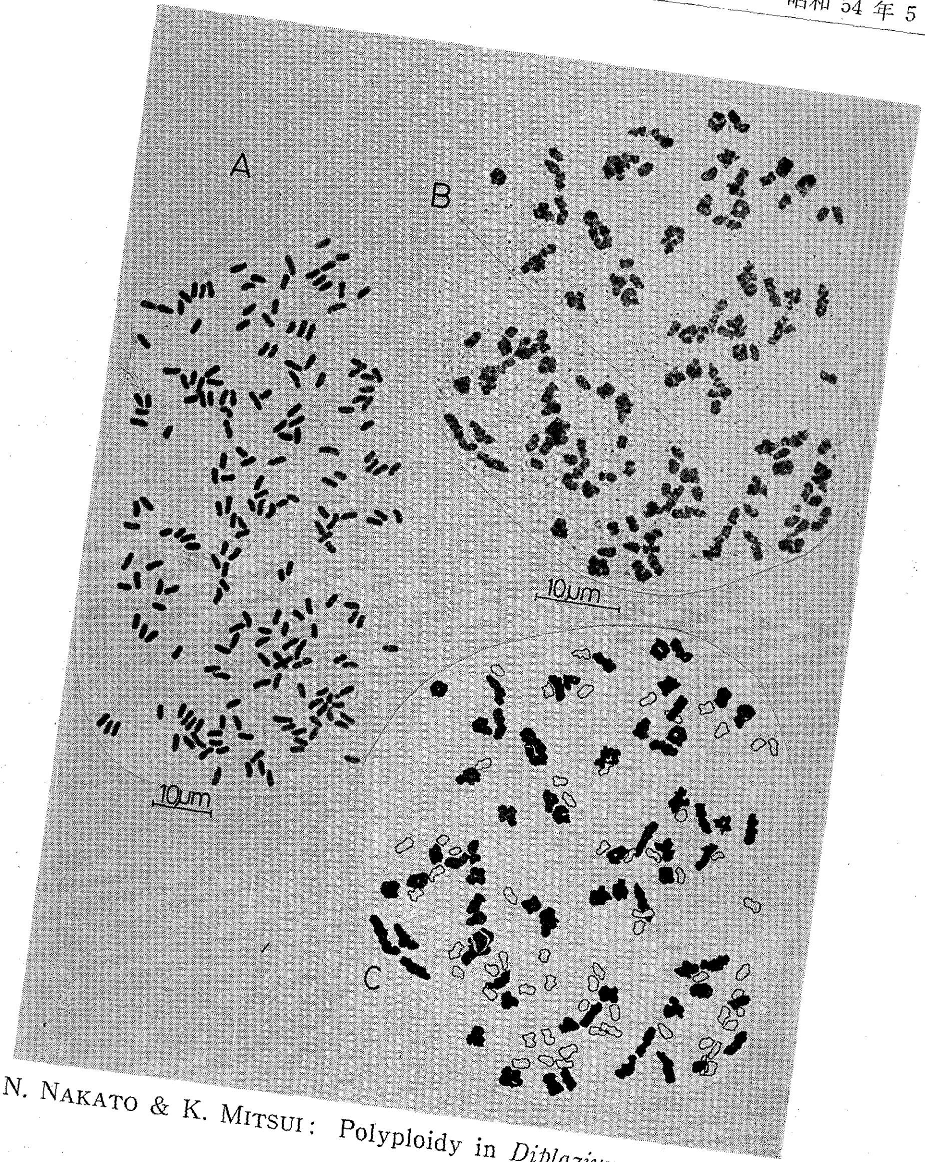

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